

shown that only phage mRNA is made in cells infected in the presence of K^+ , whereas phage mRNA and host ribosomal and mRNA are made in cells in the absence of K^+ . These data indicate, in agreement with previous results obtained by others using inhibitors of protein synthesis, that a period of protein synthesis is required after phage infection to shut off host RNA synthesis. In addition, phage mRNA can be made in the absence of simultaneous phage DNA synthesis, and without prior synthesis of phage-specific protein.

V158. Sedimentation Analysis of Replicating Phage P22 DNA. DAVID BOTSTEIN. University of Michigan Medical School, Ann Arbor.

Phage DNA synthesis was investigated during infection of *Salmonella typhimurium* by phage P22 and after induction of P22 lysogens. Sucrose gradient sedimentation analysis of gently extracted pulse-labeled DNA has revealed two intermediates in the synthesis of phage DNA. The first intermediate sediments at a rate greater than 1,000S; it contains most of the acid-precipitable 3H -thymidine incorporated during a 2-min pulse. The second intermediate sediments at a rate 1.2 to 1.5 times faster than mature phage DNA. Pulse-chase experiments indicate that the large intermediate is a precursor of the smaller one. Sedimentation in alkaline sucrose gradients suggests that in the single-stranded form the smaller intermediate still sediments faster than mature phage DNA. The large intermediate is only partially stable in alkali. After infection with ^{32}P -labeled phage, ^{32}P appears first in the large intermediate and later in the smaller one. The ratio of 3H to ^{32}P in the purified progeny of an infection with ^{32}P -labeled phage followed by pulse and chase of 3H -thymidine suggests that the DNA which is encapsulated into phage heads comes from one of the intermediates and not from DNA which sediments at the same rate as mature phage DNA.

V159. Production of Defective Particles by Induction of Lysogens of Integration Deficient Phage P22 Mutants. HAMILTON O. SMITH. University of Michigan, Ann Arbor.

Integration-deficient mutants of phage P22 have recently been described which, although unable to lyso-lyse by themselves, can become stable prophage in the host cell *Salmonella typhimurium* by complementation with wild-type phage. These mutant lysogens, when induced, yield predominantly defective particles. The density of the defective particles in CsCl varies slightly (in either direction) from that of infective phage depending on the particular mutant used. In the one case so far examined, the DNA is also altered in density; this could account for the observed shift in defective-particle density. No deviation from normal size of the DNA has been detected on sucrose gradients. DNA-DNA and RNA-DNA hybridization studies indicate the presence of host DNA in the defective particles. Defective lysates transduce for *proC* (adjacent to one end of the prophage) at 20 to 100 times the frequency for *proA* (adjacent to the opposite end), suggesting that the host cell DNA included in the particles is from the *proC* side. Experiments with temperature-sensitive mutants indicate that the decision for formation of normal versus defective particles is made before or just at the onset of phage DNA replication. It seems possible that these mutants affect specificity of prophage detachment.

V160. The Function of Fragments of MS2 RNA. YOSHIRO SHIMURA and DANIEL NATHANS. The Johns Hopkins University School of Medicine, Baltimore, Md.

In an attempt to relate the structure of MS2 RNA to its function as a messenger, the ability of fragments of phage RNA to direct the synthesis of specific phage proteins in *Escherichia coli* extracts is being determined. Fragments of phage RNA have been isolated from RNA-deficient particles formed in the presence of fluorouracil or by limited cleavage with ribonuclease T_1 . The RNA extracted from deficient particles had a sedimentation

rate of 19-20S, compared with 27S for normal MS2 RNA. Treatment of intact RNA with T_1 first yielded fragments with S values of approximately 20 and 14 in EDTA-phosphate buffer. With increasing time of reaction, the 20S fragment disappeared and a broad 14S peak resulted. Whereas intact MS2 RNA directed the synthesis of three electrophoretically separable proteins, the RNA fragment extracted from fluorouracil particles directed the synthesis of only two of these proteins. The missing product has been tentatively identified as the phage-specific RNA synthetase.

V161. Effect of RNA Bacteriophage Infection on Polyribosomal Distributions in *Escherichia coli*. B. H. IGLEWSKI and R. M. FRANKLIN. Public Health Research Institute of the City of New York, Inc., New York, N.Y.

The objective of this investigation was to examine possible alterations in distributions of bacterial polyribosomes after infection with bacteriophage R17. Polyribosomes were prepared from *Escherichia coli* 3000 by treating whole cells with lysozyme for 6 min, followed by osmotic lysing in the presence of Brij 58 plus deoxyribonuclease. The total lysate was layered on a 10 to 40% linear sucrose gradient and centrifuged for 195 min at 25,000 rev/min in the SW 25.3 rotor. The cells were labeled for 3 min with $1 \mu c$ per ml of a mixture of ^{14}C amino acids and for 30 sec with $10 \mu c$ per ml of 3H uridine. Employing this procedure, polyribosomes containing two, three, four, five, and six ribosomes were clearly resolved. Furthermore, between 70 and 80% of the ribosomal material from uninfected cells sedimented as polyribosomes. Following 10 min of infection with R17, there was a noticeable decrease in the OD₂₆₀ in the region of the heaviest polyribosomes. At this time there was no significant alteration in the labeling patterns of either nascent protein or incorporated 3H -uridine. After 25 min of virus infection there was an even greater decrease in the amount of polyribosomes. There was also a significant decrease in the amount of incorporated 3H -uridine in the area of the polyribosomes. Whereas the incorporation of ^{14}C amino acids was minimal in the region of the larger polyribosomes, there was a clearly defined peak of nascent protein in the region of two, three, and four ribosomes. After 40 min of infection there was still a decrease in the concentration of polyribosomes observed both in the OD pattern and in the amount of incorporation of labeled precursors in this region as compared to uninfected controls.

V162. Amino Acid Biosynthesis of *Streptomyces griseus* Phage. S. S. NISHIKAWA and C. M. GILMOUR. Oregon State University, Corvallis.

Biosynthesis of the component amino acids of *Streptomyces griseus* phage protein was studied by use of appropriate chromatographic and ^{14}C -labeling techniques. A complete amino acid analysis on the purified phage protein was determined with the Spino Amino Acid Analyzer 120B. Radioactivity of acidic amino acids was obtained by employing a Packard Flow Monitor Series 320E which was connected to the amino acid analyzer. Labeling patterns of both the host and phage amino acids derived from glucose-1- and 6- ^{14}C were compared and indicated involvement of both the EMP and pentose pathways. Examination of the labeling patterns in the host protein indicated that the amino acids are synthesized primarily via glycolysis. A control culture with pronounced pentose pathway activity gave expected amino acid labeling pattern in which much higher amino acid-specific activities were obtained via glucose-6- ^{14}C -grown cells. A second control culture with a predominant glycolytic pathway showed a 1:1.5 distribution pattern with glucose-1 and -6, respectively. Thus, the use of the specific activities of the obtained amino acids does provide a valid index of the route of carbohydrate catabolism used for synthesis of phage amino acids.